

What is claimed is:

1. A polynucleotide encoding anti-freeze protein, comprising a nucleotide sequence represented by SEQ ID NO:1.

5 2. A nucleotide construct composed, in the following order, of a nucleotide sequence encoding anti-freeze protein comprising nucleotide sequence represented by SEQ ID NO:1, protease cleavage site, multiple cloning site comprising sites recognized by plural restriction enzymes, and stop codon.

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3. A nucleotide construct composed, in the following order, of multiple cloning site comprising sites recognized by plural restriction enzymes, protease cleavage site, a nucleotide sequence encoding anti-freeze protein comprising nucleotide sequence
15 represented by SEQ ID NO:1, and stop codon.

4. The nucleotide construct according to claim 2 or 3, wherein said multiple cloning site comprises at least two recognition sites selected from the group consisting of *NcoI*, *XbaI*, and *BamHI*.

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5. The nucleotide construct according to claim 2, wherein said protease cleavage site is enterokinase cleavage site.

6. The nucleotide construct according to claim 3, wherein said
25 protease cleavage site is thrombin cleavage site.

7. The nucleotide construct according to claim 2 or 3, wherein said stop codon is TAG.

30 8. The nucleotide construct according to claim 2, wherein said nucleotide construct comprises a nucleotide sequence represented

by SEQ ID NO:2.

9. The nucleotide construct according to claim 3, wherein said nucleotide construct comprises a nucleotide sequence represented
5 by SEQ ID NO:3.

10. An expression vector for plant comprising (i) the nucleotide construct according to claim 2 or 3, wherein a nucleotide sequence encoding a target protein is inserted into the multiple cloning
10 site; (ii) a promoter that functions in plant cells to cause the production of an RNA molecule operably linked to the nucleotide construct of (i); and (iii) a 3'-non-translated region that functions in plant cells to cause the polyadenylation of the 3'-end of said RNA molecule.

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11. A method for preparing a transient transfected plant expressing a recombinant protein transiently, which comprises the steps of:

- (a) introducing the plant expression vector according to claim
20 10 into a plant cell; and
(b) confirming whether the gene has been introduced into said plant cell.

12. A transient transfected plant prepared by the method according
25 to claim 11, expressing the recombinant protein transiently.

13. A method for producing a recombinant protein by using a transient transgenic plant as a bioreactor, which comprises the steps of:

- 30 (a) introducing the plant expression vector according to claim 10 into a plant cell;

(b) confirming whether the gene has been introduced into said plant cell; and

(c) obtaining the recombinant protein from a plant comprising the plant cell introduced with the gene.

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14. A method for preparing a transgenic plant expressing a recombinant protein stably, which comprises the steps of:

(a) introducing the expression vector for plant according to claim 10 into a plant cell;

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(b) selecting a transformed plant cell; and

(c) regenerating a plant from the transformed plant cell to obtain a transgenic plant.

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15. A transgenic plant prepared by the method according to claim 14, expressing the recombinant protein stably.

16. A method for producing a recombinant protein by using a transgenic plant as a bioreactor, which comprises the steps of:

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(a) introducing the expression vector for plant according to claim 10 into a plant cell;

(b) selecting a transformed plant cell;

(c) regenerating a plant from the transformed plant cell to obtain a transgenic plant; and

(d) obtaining the recombinant protein from the transgenic plant.

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17. A recombinant protein produced by the method according to claim 13 or 16.

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18. The method according to claim 13 or 16, said step of obtaining the recombinant protein is performed by using an ice-filled column.

19. The method according to claim 13 or 16, said step of obtaining the recombinant protein is performed by using an ice-nucleation material comprising silver iodide crystal or alive or dead microorganism, *Pseudomonas syringae*.

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20. The method according to claim 13 or 16, said step of obtaining the recombinant protein is performed by using a hypertonic solution comprising monosaccharides, disaccharides, polysaccharides or sugar-alcohol.

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21. The method according to claim 13 or 16, said step of obtaining the recombinant protein is performed by using a freeze-control device equipped with a low temperature controller and a stirrer, capable of controlling freezing-rate.

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22. The method according to claim 19 or 20, wherein said method further uses a freeze-control device equipped with equipped a low temperature controller and a stirrer, capable of controlling freezing-rate.

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23. A method for isolating AFP-fused recombinant protein, which comprises the step of;

(a) contacting to ice crystal a recombinant fusion protein comprising target protein and AFP; and

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(b) recovering the ice crystal to which the recombinant protein is attached.

24. The method according to claim 23, wherein said AFP is derived from plants, fungi or fishes.

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25. The method according to claim 23, said AFP corresponds to the

ice crystal-attaching domain of the full length of AFP amino acid sequence.

26. The method according to claim 23, wherein said recombinant
5 protein is produced by the method for preparing a transgenic plant
expressing the recombinant protein, which comprises the steps of;

- (a) preparing an expression vector comprising a construct in
which a nucleotide sequence encoding AFP are linked to 5'-end
or 3'-end of a nucleotide sequence encoding a target protein
10 and protease cleavage site exists between the target protein-
coding sequence and AFP-coding sequence;
- (b) introducing the expression vector into a host; and
- (c) selecting a transformed host.

15 27. The method according to claim 26, wherein said protease
cleavage site is enterokinase cleavage site.

28. The method according to claim 26, wherein said expression
vector is an expression vector for plant, animal or microorganism.

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29. The method according to claim 26, wherein said host is a cell
of plant, animal or microorganism, a plant or an animal.